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GPR56/ADGRG1 Inhibits Mesenchymal Differentiation and Radioresistance in Glioblastoma

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Skin accumulation of advanced glycation end products is increased in patients with an abdominal aortic aneurysm



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ABSTRACT

Objective: Advanced glycation end products (AGEs) are implicated in the pathogenesis of cardiovascular disease. Accumulation of AGEs is driven by oxidative or glycemic stress and can be assessed by skin autofluorescence (SAF). SAF is increased in patients with peripheral artery disease (PAD) and independently associated with mortality and major adverse cardiovascular events in these patients. PAD and abdominal aortic aneurysm (AAA) share several risk factors. Inflammation is an important process in AAA formation and increases levels of oxidative stress. We therefore hypothesized that SAF would be increased in AAA patients compared with controls.

Methods: A case-control study was performed in 248 AAA patients and 124 controls without AAA or PAD matched for age and presence of diabetes mellitus. SAF was noninvasively assessed with the AGE Reader (Diagnoptics Technologies BV, Groningen, The Netherlands).

Results: SAF was higher in AAA patients than in controls: 2.89 ± 0.63 vs 2.68 ± 0.63 arbitrary units ($P = .003$). PAD comorbidity was associated with increased SAF within the AAA patient group ($P = .01$). After correction for known factors influencing SAF (age, current smoking, hypertension, and estimated glomerular filtration rate), PAD comorbidity remained an independent determinant of SAF. Logistic regression analysis of the total cohort showed an unadjusted odds ratio (OR) of 1.74 (95% confidence interval [CI], 1.20-2.51) for the presence of AAA with each unit increase of SAF and an adjusted OR of 1.78 (95% CI, 1.22-2.60) after correction for cardiovascular comorbidity (cerebrovascular disease and coronary artery disease). After additional correction for sex, current smoking, hypertension, and use of lipid-lowering drugs, this significance was lost (adjusted OR, 1.53; 95% CI, 0.94-2.48).

Conclusions: Skin accumulation of AGEs, measured by SAF, is increased in patients with AAA compared with controls without AAA or PAD, independent of the presence of coronary artery disease and cerebrovascular disease. In AAA patients, SAF is closely associated with the presence of PAD and cardiovascular risk factors. (J Vasc Surg 2017;66:1696-703.)

An abdominal aortic aneurysm (AAA) is characterized by enlargement of the aorta as a consequence of pathophysiological changes in the aortic vascular wall, including inflammation, smooth muscle cell apoptosis, and proteolysis of elastin and collagen in the tunica media.¹ Most AAAs are discovered as an incidental finding because this disease is asymptomatic in most

patients.² Rupture of the AAA is generally an emergency situation with an estimated mortality rate of 80%.³

Advanced glycation end products (AGEs) are formed by nonenzymatic reactions on reducing sugars and proteins and have two potentially harmful effects.⁴ First, formation of cross-links contributes to stiffening of the myocardium and arteries.⁵ Second, interaction with the receptor for AGEs induces inflammatory responses through activation of nuclear factor- κ B and consecutive release of proinflammatory cytokines.⁴

Assessment of skin AGEs can be performed with a noninvasive technique called skin autofluorescence (SAF).⁶ The estimated turnover time of skin collagen is 15 years; thus, skin AGEs and SAF represent a long-term metabolic memory.⁷ In addition, SAF is positively related to AGE levels in cardiac tissue and venous bypass graft material, both vascular tissues with also a typical slow turnover.^{8,9}

Although the formation and accumulation of AGEs in long-lived tissues occurs physiologically with aging, this formation is enhanced in conditions associated with hyperglycemia or oxidative stress such as diabetes mellitus (DM), autoimmune disease, renal insufficiency, and atherosclerosis.⁴ As a result, SAF is increased in patients with these conditions compared with controls,¹⁰⁻¹³ and increased SAF is also associated with major adverse

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Author conflict of interest: A.J.S. is founder and shareholder of Diagnoptics BV, The Netherlands, manufacturing autofluorescence readers (<http://diagnoptics.com>).

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cardiovascular events in patients with DM, myocardial infarction, and peripheral artery disease (PAD).¹⁴⁻¹⁷

PAD and AAA disease share several risk factors and are frequently found in the same patient. Inflammation is an important pathway of AAA formation, mediating elastin and collagen degradation by proteases derived from inflammatory cells.¹⁸ This led us to hypothesize that SAF, as a measure of skin AGEs, would be increased in patients with an AAA; therefore, the aim of this study was to compare SAF levels in AAA patients and controls. We also investigated whether SAF is associated with the presence of AAA after correction for cardiovascular risk factors, such as smoking, and cardiovascular comorbidity, expressed by a history of cerebrovascular disease (CVD) or coronary artery disease (CAD).

METHODS

Study population. We performed a case-control study. AAA patients were recruited from the vascular surgery outpatient clinic at the University Medical Center Groningen (UMCG), The Netherlands, between 2007 and 2011. Patients at least 18 years of age with a confirmed AAA were eligible to participate. AAA was ascertained by evidence of an enlarged diameter of the aorta of ≥ 30 mm on ultrasound imaging, magnetic resonance angiography, or computed tomography angiography. In case of an emergency or elective repair without available medical imaging reports, surgical reports or referral letters were used to confirm the diagnosis.

AAA patients and controls were matched 2:1 by age and presence of DM (Fig 1). Controls were selected from earlier studies. Diabetic controls were selected from a cohort ($n = 973$) from the diabetes outpatient clinic, Zwolle, The Netherlands.¹¹ The nondiabetic control group consisted of patients admitted to the UMCG for surgical interventions ($n = 231$) unrelated to cardiovascular disease and of patients who visited the vascular surgery outpatient clinic in the UMCG ($n = 121$), mostly because of varicose veins or carotid artery stenosis, who had no history or symptoms of AAA or PAD.^{12,13}

Exclusion criteria for AAA patients and controls were sepsis, recent myocardial infarction or stroke, defined as an event ≤ 3 months before recruitment, renal replacement therapy or end-stage renal disease (estimated glomerular filtration rate [eGFR] < 15 mL/min/1.73m²), solid organ transplantation, or active cancer. AAA patients with a mycotic or inflammatory aneurysm or a history of connective tissue disease were excluded. Control patients with a history or symptoms of AAA or PAD were also excluded.

All participating patients gave informed consent. The study was approved by the local Institutional Review Board and complied with the principles of the Declaration of Helsinki.

ARTICLE HIGHLIGHTS

- **Type of Research:** Single-center prospective case-controlled study
- **Take Home Message:** Skin accumulation of advanced glycation end products as measured by skin autofluorescence was increased in 248 patients with abdominal aortic aneurysm compared with 124 matched controls.
- **Recommendation:** This study suggests that using skin autofluorescence to measure advanced glycation end products may be a useful in the detection of patients with abdominal aortic aneurysm.

Data collection. Traditional cardiovascular risk factors were prospectively assessed, including current smoking status, body mass index, presence of DM, hypertension, eGFR, and use of lipid-lowering, glucose-lowering, or anticoagulant drugs. eGFR was calculated from the serum creatinine level using the Modification of Diet in Renal Disease formula.¹⁹ Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg, a diastolic blood pressure ≥ 90 mm Hg, or the use of blood pressure-lowering drugs. Lipid-lowering therapy was defined as the use of statins, ezetimibe, or fibrates. For glucose-lowering therapy, the use of metformin, dipeptidyl peptidase 4 inhibitors, repaglinide, sulfonylurea derivatives, pioglitazone, glucagon-like peptide 1 receptor agonists, sodium-glucose cotransporter 2 inhibitors, and insulin was assessed. Anticoagulant therapy was defined as the use of anticoagulant or antiplatelet therapy.

History of cardiovascular comorbidity was retrieved from medical records and divided into CAD, CVD, and PAD. CAD was defined as a history of angina pectoris, myocardial infarction, percutaneous coronary intervention, or coronary artery bypass graft. CVD was defined as a history of stroke, transient ischemic attack, carotid endarterectomy, or carotid stenting. PAD was defined as a resting ankle-brachial index ≤ 0.90 combined with confirmation of obstructive disease on computed tomography angiography, magnetic resonance angiography, catheter angiography, or duplex ultrasound imaging.

For AAA patients, data on the diameter, surgical repair, and rupture of the aneurysm were obtained. The presence of aneurysms in other locations in addition to the abdominal aorta was assessed. Rupture of the AAA was confirmed using ultrasound imaging or during surgical repair of the aneurysm.

SAF assessment. SAF as a noninvasive measure of skin AGEs was assessed using the AGE Reader (Diagnostics Technologies BV, Groningen, The Netherlands). This device uses ultraviolet A light to measure the dermal content of certain AGEs using their fluorescent properties.

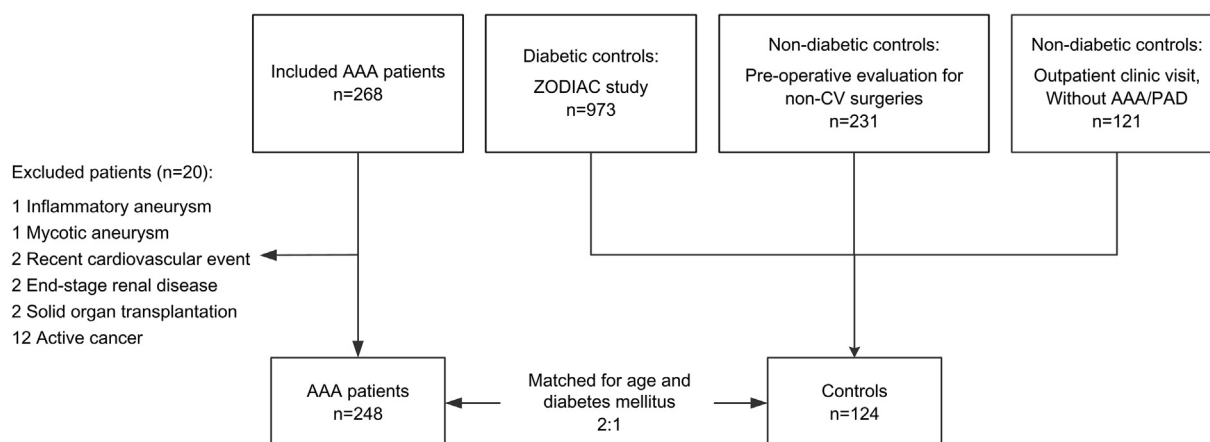


Fig 1. Flowchart of the study inclusion process of patients with abdominal aortic aneurysm (AAA) patients and controls. CV, Cardiovascular; PAD, peripheral artery disease; ZODIAC, Zwolle Outpatient Diabetes project Integrating Available Care.

An area of 4 cm² on the inner forearm is shielded from surrounding light and exposed to excitation light with a wavelength between 300 and 420 nm with a peak of 370 nm.⁶ Fluorescent AGEs characteristically emit light of a wavelength between 420 and 600 nm with a peak of ~440 to 450 nm, whereas reflected light is identical to the excitation light. SAF is calculated as the ratio between emission light and reflected light, multiplied by 100 and expressed in arbitrary units.

Previous studies by Meerwaldt et al^{6,20} showed a strong correlation between SAF and skin biopsy specimen content of several important AGEs, including the fluorescent AGE pentosidine and two nonfluorescent AGEs, *N*-carboxy-methyl-lysine and *N*-carboxy-ethyl-lysine. The inner forearm was accepted as the standard and most accessible location to measure SAF because a validation study showed a strong correlation between measurements of the arm and the leg.⁶ The mean SAF is calculated from three consecutive measurements performed automatically by the AGE Reader. An intraindividual variance of 4.5% was shown in an earlier study with measurements performed on a single day.²¹

Statistical analysis. Data are shown as mean \pm standard deviation, median (interquartile range [IQR]), or as number (%). Characteristics of AAA patients and controls were compared using the Student independent *t*-test or the χ^2 test. SAF between groups was compared using the Student independent *t*-test.

The determinants of SAF in the AAA patients were evaluated in a backward multivariable linear regression analysis. A *P* value of $<.05$ was considered statistically significant. To study the association between SAF and the presence of AAA, a logistic regression analysis was performed in the complete study group (AAA patients and controls). Three models were tested: a crude model with SAF as the sole determinant, an adjusted model 1 with SAF and cardiovascular comorbidity (presence of

CVD and CAD), and an adjusted model 2 with SAF, cardiovascular comorbidity, and risk factors of AAA (ie, sex, current smoking, hypertension and use of lipid-lowering drugs). The logistic regression models were intrinsically adjusted for the matched variables, (age and DM). PAD was not included in the models because the controls were selected on the absence of PAD. The statistical analyses were performed using SPSS software version 22.0 (IBM, Armonk, NY).

RESULTS

Characteristics of patients. Of the 268 patients with AAA who were eligible to participate, 20 were excluded due to an inflammatory ($n = 1$) or mycotic aneurysm ($n = 1$), recent cardiovascular event ($n = 2$), end-stage renal disease ($n = 2$), solid-organ transplantation ($n = 2$), or active cancer ($n = 12$; Fig 1). The remaining 248 AAA patients were matched for age and presence of DM with 124 controls.

Surgical repair of the aneurysm had been performed in 160 AAA patients (65%), 27 (11%) of which were performed because of rupture of the aneurysm. The median preoperative diameter of the aneurysm was 60 mm (IQR, 55-70 mm). In the patients who had not undergone surgical repair, the median diameter was 48 mm (IQR, 42-57 mm). The diameter of the aorta was not measured preoperatively in 26 patients (10%) due to emergency repair of a ruptured AAA ($n = 9$) or because the operation was performed in a different hospital without traceable information on diameter ($n = 17$). Median time between surgery and inclusion date was 3.20 years (IQR, 1.00-7.39 years). In nine AAA patients (4%), simultaneous aneurysms were found in other locations in addition to the abdominal aorta, comprising the iliac artery ($n = 4$), popliteal artery ($n = 2$), thoracic aorta ($n = 1$), femoral artery ($n = 1$), and carotid artery ($n = 1$).

Characteristics of the AAA patients and controls are summarized in Table 1. Patients and controls had a

Table I. Baseline characteristics of abdominal aortic aneurysm (AAA) patients and controls^a

Characteristics ^b	AAA patients (n = 248)	Controls (n = 124)	P value
Age, years	72.9 ± 7.1	73.0 ± 7.0	Matched
Male sex	227 (92)	51 (41)	<.0001
Current smoker	82 (33)	18 (15)	<.0001
Body mass index, ^c kg/m ²	27.2 ± 4.3	27.7 ± 4.9	NS
DM	41 (17)	21 (17)	Matched
Blood pressure			
Systolic, ^d mm Hg	139.1 ± 22.1	154.2 ± 24.5	<.0001
Diastolic, ^e mm Hg	78.4 ± 13.1	81.4 ± 9.9	.013
Hypertension	234 (94)	112 (90)	NS
Medication use			
Lipid-lowering drugs	181 (73)	35 (28)	<.0001
Glucose-lowering drugs	31 (13)	... ^f	
Anticoagulant therapy	216 (87)	38 (31)	<.0001
eGFR, ^g mL/min/1.73m ²	72.4 ± 22.5	68.0 ± 17.5	.044
Cardiovascular comorbidity			
CAD	115 (46)	16 (13)	<.0001
CVD	42 (17)	17 (14)	NS
PAD	77 (31)	0 (0)	<.0001
AAA diameters, mm			
Patients without surgical repair	48 (42-57)	N/A	
Patients with surgical repair ^h	60 (55-70)	N/A	
Surgical repair of AAA	160 (65)	N/A	
EVAR	87 (35)	N/A	
Open repair	73 (29)	N/A	
Rupture of AAA	27 (11)	N/A	
Skin autofluorescence, AU	2.89 ± 0.63	2.68 ± 0.63	.003

AU, Arbitrary units; CAD, coronary artery disease; CVD, cerebrovascular disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; EVAR, endovascular aneurysm repair; NS, not significant; N/A, not applicable; PAD, peripheral artery disease.

^aAAA patients and controls were matched 2:1 on age and diabetes.

^bData are presented as number of patients (%), mean ± standard deviation, or median (interquartile range [IQR]).

^cData missing for seven patients.

^dData missing for five patients.

^eData missing for five patients.

^fData missing for all diabetic controls (n = 21).

^gData missing for 11 patients.

^hDiameter of AAA before surgery in patients with surgical repair, missing for 26 patients.

mean age of 73 ± 7 years (range, 55-90 years in AAA patients and 57-90 years in controls). A higher proportion of AAA patients were men compared with controls (92% vs 41%; $P < .001$). DM was present in 17% of the AAA patients and controls. Occurrence of hypertension did not differ between groups, but the mean systolic and diastolic blood pressures were lower in the AAA patient group than in the control group ($P < .0001$ and $P = .013$, respectively). The use of lipid-lowering and anticoagulant drugs was higher in the AAA patients than in the controls ($P < .0001$ for both). CAD and PAD were more common in AAA patients ($P < .0001$ for both), but the proportion of patients with CVD did not differ.

SAF results. SAF was significantly higher in AAA patients vs controls, at 2.89 ± 0.63 vs 2.68 ± 0.63 arbitrary

units ($P = .003$; Table I; Fig 2). Within the AAA patients, SAF was higher in the patients with PAD than in the patients without PAD ($P = .01$ by univariable analysis; Fig 2). In a multivariable analysis of SAF, PAD remained a significant determinant (Table II).

In the AAA patients, multivariable linear regression analysis showed that age, current smoking, hypertension, eGFR, and PAD were significant determinants of SAF (adjusted $R^2 = 0.10$; Table II). Sex, presence of DM, body mass index, use of lipid-lowering drugs, anticoagulant therapy, aortic diameter, and a history of CAD or CVD were not determinants of SAF in the AAA patients. SAF levels did not differ between AAA patients who had not undergone surgical repair, AAA patients who had undergone open repair, and AAA patients who had undergone endovascular repair (Supplementary Fig, online only).

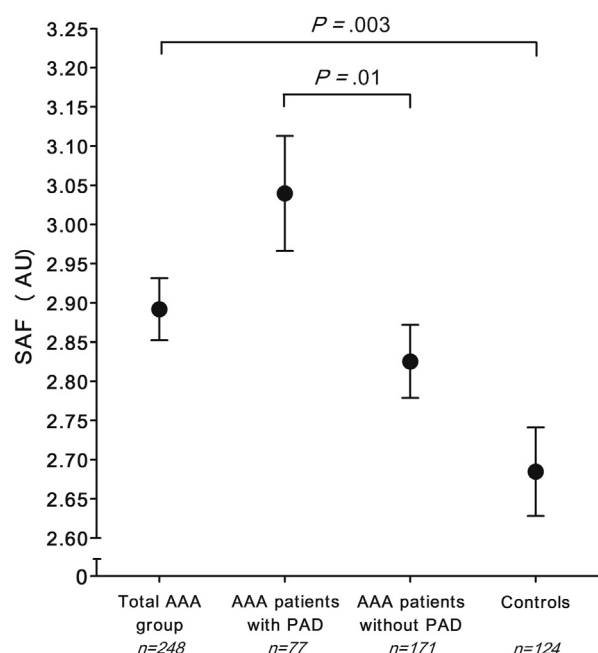


Fig 2. Skin autofluorescence (SAF) in abdominal aortic aneurysm (AAA) patients, grouped by those with and without peripheral artery disease (PAD), and controls. Data are shown in arbitrary units (AU) as the mean \pm standard error of the mean (range bars). SAF is higher in the total group of AAA patients than in the controls: 2.89 ± 0.04 vs 2.68 ± 0.06 ($P = .003$ by Student independent *t*-test). SAF is higher in the AAA patients with PAD than in the AAA patients without PAD: 3.04 ± 0.07 vs 2.83 ± 0.05 ($P = .01$ by Student independent *t*-test).

Table III reports the result of the logistic regression models that describe the association between SAF and the presence of AAA in the total cohort. In the crude model, SAF was associated with presence of AAA (odds ratio [OR], 1.74; 95% confidence interval [CI], 1.20-2.51). After correction for cardiovascular comorbidity (presence of CAD or CVD), this association remained significant (OR, 1.78; 95% CI, 1.22-2.60; adjusted model 1). After additional correction for cardiovascular risk factors in adjusted model 2 (sex, current smoking, hypertension and use of lipid-lowering drugs), the association lost its significance (OR, 1.53; 95% CI, 0.94-2.48).

DISCUSSION

This study shows that SAF, as a measure of skin accumulation of AGEs, is increased in patients with AAA compared with controls. Furthermore, the association of SAF with AAA disease is independent of the presence of cardiovascular comorbidity. Still, within the AAA patients, SAF is closely associated with the presence of PAD and cardiovascular risk factors.

In several cardiovascular risk groups and disease, such as renal insufficiency, DM, CAD, and PAD, AGE levels are increased compared with controls.^{12,15,20,22} The present study adds AAA patients as a group at high cardiovascular risk with increased accumulation of AGEs. We found

the association between SAF and AAA to be independent of the presence of CAD and CVD. Earlier studies on the association between AAA and AGEs are scarce. A study of eight AAA patients found that AGEs and receptor for AGE in the aortic wall were increased compared with five control samples from a tissue bank of autopsy materials.²³ The same report described a reduction in the incidence of AAA of 50% in AGE receptor-knockout mice compared with control mice.²³ In contrast, another study of 60 AAA patients with and without DM showed a lower pentoside content in the aortic aneurysm wall compared with 26 aortic control samples with and without DM.²⁴ These differences in results may be explained by the type of AGEs that were measured; the latter study only measured the cross-linking AGE pentosidine, whereas the former study measured total AGEs content. In our present study, we used SAF to assess AGEs accumulation, which is correlated to the level of both cross-linking and noncross-linking AGEs.⁶ Another explanation for the discrepancy between the above-mentioned studies may be the nature of the control samples, since these were taken from autopsy specimens. Whether accumulation of AGEs changes after death is unknown. Finally, it is possible that AGEs exert a double-sided effect on AAA development. On the one hand, AGEs might stimulate AAA progression by inducing endothelial dysfunction, calcification, loss of compliance, and thrombogenesis. On the other hand, cross-linking AGEs might have a protective effect on aneurysm growth through stiffening of the vascular wall. A recent study in a mouse AAA model confirmed that homogenous aortic stiffening reduces aneurysm growth.²⁵

The association we found between SAF and AAA was partly explained by the presence of cardiovascular risk factors. Although smoking is a confounding factor in this analysis, contributing to the generation and accumulation of AGEs while also being a known risk factor for AAA, this cannot fully negate this interaction.^{2,26} Therefore, we concluded that SAF in AAA patients is closely associated with cardiovascular risk factors and coexisting PAD rather than being solely associated with the presence of AAA disease. This is in line with earlier reports on increased SAF in PAD patients and increased SAF in patients with carotid artery disease that was mainly explained by the coexistence of PAD.^{12,27}

Interestingly, we found a lower prevalence of DM in AAA patients than in our earlier report on PAD patients.¹² This observation is in line with a systematic review showing a reduced rate of diabetes in AAA patients.²⁸ Possibly, diabetes exhibits a protective effect on AAA development through increased arterial stiffness induced by cross-linking of AGEs. In the earlier mentioned study, AAA samples of diabetic patients showed a higher level of the cross-linking AGE pentosidine than AAA samples from nondiabetic individuals,

Table II. Multivariable linear regression analysis of skin autofluorescence (SAF) in abdominal aortic aneurysm (AAA) patients

Variables ^a	Coefficient β	SE	Standardized coefficient β	P value
Age	0.014	0.006	0.157	.019
Current smoking	0.280	0.087	0.215	.001
Hypertension	−0.411	0.189	−0.141	.031
eGFR (mL/min/1.73m ²)	−0.004	0.002	−0.136	.037
PAD	0.196	0.085	0.148	.022

eGFR, Estimated glomerular filtration rate; PAD, peripheral artery disease; SE, standard error.
^aVariables removed from the model were sex, diabetes mellitus (DM), body mass index, anticoagulant therapy, use of lipid-lowering drugs, aortic diameter, coronary artery disease (CAD) and cerebrovascular disease (CVD).

Table III. Association between abdominal aortic aneurysm (AAA) disease and skin autofluorescence (SAF) in the total study group^a

Variable	OR	95% CI
Crude model		
SAF	1.74	1.20-2.51
Adjusted model 1		
SAF	1.78	1.22-2.60
CAD	6.40	3.51-11.67
CVD	1.11	0.57-2.17
Adjusted model 2		
SAF	1.53	0.94-2.48
Male sex ^b	16.57	8.15-33.70
Current smoking	3.39	1.61-7.13
Hypertension	2.08	0.73-5.95
Use of lipid-lowering drugs	6.94	3.63-13.29
CAD	2.79	1.36-5.75
CVD	0.43	0.19-0.99

CAD, Coronary artery disease; CI, confidence interval; CVD, cerebrovascular disease; OR, odds ratio.
^aAll crude and adjusted logistic regression models were intrinsically adjusted for the matched variables of age and diabetes mellitus (DM). The model did not include peripheral artery disease (PAD) because controls were selected on the absence of PAD.
^bFemale is the reference.

which correlated negatively with AAA diameter.²⁴ To further elucidate the possible role of AGEs in the inverse association of DM with atherosclerotic diseases vs AAA, we designed the ARTERY (Advanced glycation end-products in patients with peripheral artery disease and abdominal aortic aneurysm) study.²⁹ In this study, AGEs will be measured in aortic and macrovascular waste material in diabetic and nondiabetic subjects with AAA or PAD.

Although we showed that SAF is increased in AAA patients and remains associated with AAA disease after correction for cardiovascular comorbidity, more research is needed before conclusions can be drawn about the clinical implications of SAF measurements in AAA disease. Specifically, an investigation of the association between SAF and mortality, cardiovascular events, and growth or rupture of the aneurysm in AAA patients is necessary.¹⁵⁻¹⁷ Still, our findings contribute to the scarce

knowledge available on AGEs accumulation in AAA disease.

Our study has several limitations. Firstly, the large proportion of patients who underwent surgical repair of the AAA before inclusion into the study shows that our population already had extensive disease. Also, the proportion of patients with CAD or CVD illustrates widespread atherosclerosis in these patients. Thus, our findings may not be representative for earlier stages of AAA.

Secondly, our controls were selected on the absence of a history and symptoms of AAA or PAD, whereas a proportion of our AAA patients did have concurrent PAD. Therefore, the comparison of SAF between AAA patients and controls could not be corrected for the presence of PAD. However, our multivariable linear regression analysis of SAF in the AAA patients showed that PAD is an important determinant. Although it is possible that a small proportion of our control subjects had undiscovered AAA or PAD disease, this would lead to an underestimation of the effect of SAF on AAA presence rather than an overestimation.

Thirdly, the cross-sectional design of this study did not permit conclusions to be drawn about the causality of the observed associations.

Fourthly, a limitation of the applicability of the AGE Reader is that it can only be used on individuals with skin pigmentation up to Fitzpatrick type V. In skin with higher pigmentation, excitation and emission light are both absorbed excessively by melanin, resulting in inaccurate SAF measurements.

Finally, levels of AGEs were only assessed by SAF and not directly in vascular tissues. The association between SAF and AGE content of human blood vessels has been investigated in patients with CAD, showing that SAF is correlated with the AGE content of cardiac tissue and venous bypass graft material.^{8,9} This grants probability to the possible association between SAF and AGEs in the human arterial wall in AAA patients.

CONCLUSIONS

SAF, as a noninvasive assessment of skin accumulation of AGEs, is increased in patients with AAA compared with controls, independent of cardiovascular comorbidity.

Still, within the AAA patients, SAF is closely associated with the presence of PAD and cardiovascular risk factors. These findings suggest that accumulation of AGEs is increased in AAA patients, partly explained by presence of widespread atherosclerosis.

AUTHOR CONTRIBUTIONS

Conception and design: JL

Analysis and interpretation: JB, LdV, TL, AS, CZ, JL

Data collection: JB, LdV, DM

Writing the article: JB, LdV, JL

Critical revision of the article: JB, LdV, TL, DM, AS, CZ, JL

Final approval of the article: JB, LdV, TL, DM, AS, CZ, JL

Statistical analysis: JB, LdV

Obtained funding: Not applicable

Overall responsibility: JL

REFERENCES

- Nordon IM, Hinchliffe RJ, Loftus IM, Thompson MM. Pathophysiology and epidemiology of abdominal aortic aneurysms. *Nat Rev Cardiol* 2011;8:92-102.
- Erbel R, Aboyans V, Boileau C, Bossone E, Bartolomeo RD, Eggebrecht H, et al. 2014 ESC guidelines on the diagnosis and treatment of aortic diseases: document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The Task Force for the Diagnosis and Treatment of Aortic Diseases of the European Society of Cardiology (ESC). *Eur Heart J* 2014;35:2873-926.
- Reimerink JJ, van der Laan MJ, Koelemay MJ, Balm R, Legemate DA. Systematic review and meta-analysis of population-based mortality from ruptured abdominal aortic aneurysm. *Br J Surg* 2013;100:1405-13.
- Goldin A, Beckmann JA, Schmidt AM, Creager MA. Advanced glycation end products, sparking the development of diabetic vascular injury. *Circulation* 2006;114:597-605.
- Sell DR, Monnier VM. Molecular basis of arterial stiffening: role of glycation - a mini review. *Gerontology* 2012;58:227-37.
- Meerwaldt R, Graaff R, Oomen PHN, Links TP, Jager JJ, Alderson NL, et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 2004;47:1324-30.
- Verzijl N, DeGroot J, Thorpe SR, Bank RA, Shaw JN, Lyons TJ, et al. Effect of collagen turnover on the accumulation of advanced glycation end products. *J Biol Chem* 2000;275:39027-31.
- Hofmann B, Adam A, Jacobs K, Riemer M, Erbs C, Bushnaq H, et al. Advanced glycation end product associated skin autofluorescence: a mirror of vascular function? *Exp Gerontol* 2013;48:38-44.
- Hofmann B, Jacobs K, Navarrete Santos A, Wienke A, Silber RE, Simm A. Relationship between cardiac tissue glycation and skin autofluorescence in patients with coronary artery disease. *Diabetes Metab* 2015;41:410-5.
- Dadonienė J, Cypienė A, Ryliskytė L, Ruginė R, Ryliskienė K, Laucevičius A. Skin autofluorescence in systemic sclerosis is related to the disease and vascular damage: a cross-sectional analytic study of comparative groups. *Dis Markers* 2015:837470.
- Lutgers HL, Graaff R, Links TP, Ubink-Veldmaat LJ, Bilo HJ, Gans RO, et al. Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes. *Diabetes Care* 2006;29:2654-9.
- Vos de LC, Noordzij MJ, Mulder DJ, Smit AJ, Lutgers HL, Dullaart RP, et al. Skin autofluorescence as a measure of advanced glycation end products deposition is elevated in peripheral artery disease. *Arterioscler Thromb Vasc Biol* 2013;33:131-8.
- Hartog JW, de Vries AP, Lutgers HL, Meerwaldt R, Huisman RM, van Son WJ, et al. Accumulation of advanced glycation end products, measured as skin autofluorescence, in renal disease. *Ann N Y Acad Sci* 2005;1043:299-307.
- Lutgers HL, Gerrits EG, Graaff R, Links TP, Sluiter WJ, Gans RO, et al. Skin autofluorescence provides additional information to the UK Prospective Diabetes Study (UKPDS) risk score for the estimation of cardiovascular prognosis in type 2 diabetes mellitus. *Diabetologia* 2009;52:789-97.
- Mulder DJ, Haelst PL, Graaff R, Gans RO, Zijlstra F, Smit AJ. Skin autofluorescence is elevated in acute myocardial infarction and is associated with the one-year incidence of major adverse cardiac events. *Neth Heart J* 2009;17:162-8.
- de Vos LC, Mulder DJ, Smit AJ, Dullaart RP, Kleefstra N, Lijfering WM, et al. Skin autofluorescence is associated with 5-year mortality and cardiovascular events in patients with peripheral artery disease. *Arterioscler Thromb Vasc Biol* 2014;34:933-8.
- de Vos LC, Boersema J, Mulder DJ, Smit AJ, Zeebregts CJ, Lefrandt JD. Skin autofluorescence as a measure of advanced glycation end products deposition predicts 5-year amputation in patients with peripheral artery disease. *Arterioscler Thromb Vasc Biol* 2015;35:1532-7.
- McCormick ML, Gravila D, Weintraub NL. Role of oxidative stress in the pathogenesis of abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol* 2007;27:461-9.
- Levey AS, Coresh J, Greene T, Stevens LA, Zhang Y, Hendriksen S, et al. Using standardised serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006;145:247-54.
- Meerwaldt R, Hartog JW, Graaff R, Huisman RJ, Links TP, den Hollander NC, et al. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol* 2005;16:3687-93.
- Stirban A, Nandrea S, Negrean M, Koschinsky T, Tschoepe D. Skin autofluorescence increases postprandially in human subjects. *Diabetes Technol Ther* 2008;10:200-5.
- Meerwaldt R, Lutgers HL, Links TP, Graaf R, Baynes JW, Gans RO, et al. Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. *Diabetes Care* 2007;30:107-12.
- Zhang F, Kent KC, Yamanouchi D, Zhang Y, Kato K, Tsai S, et al. Anti-receptor for advanced glycation end products therapies as novel treatment for abdominal aortic aneurysm. *Ann Surg* 2009;250:416-23.
- Koole D, van Herwaarden JA, Schalkwijk CG, Lafeber FP, Vink A, Smeets MB, et al. A potential role for glycated cross-links in abdominal aortic aneurysm disease. *J Vasc Surg* 2017;65:1493-503.
- Raaz U, Zollner AM, Schellinger IN, Toh R, Nakagami F, Brandt M, et al. Segmental aortic stiffening contributes to experimental abdominal aortic aneurysm development. *Circulation* 2015;131:1783-95.
- Cerami C, Founds H, Nicholl I, Mitsuhashi T, Giordano D, Vanpatten S, et al. Tobacco smoke is a source of toxic reactive glycation products. *Proc Natl Acad Sci U S A* 1997;94:13915-20.
- Noordzij MJ, Lefrandt JD, Loeffen EA, Saleem BR, Meerwaldt R, Lutgers HL, et al. Skin autofluorescence is increased in patients with carotid artery stenosis and

- peripheral artery disease. *Int J Cardiovasc Imaging* 2012;28:431-8.
28. Shantikumar S, Ajjan R, Porter KE, Scott DJ. Diabetes and the abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* 2010;39:200-7.
29. de Vos LC, Boersema J, Hillebrands JL, Schalkwijk CG, Meerwaldt R, Breek JC, et al. Diverging effects of diabetes mellitus in patients with peripheral artery disease and abdominal aortic aneurysm and the role of advanced

glycation end-products: ARTERY study – protocol for a multicentre cross-sectional study. *BMJ Open* 2017;7:e012584.

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